The Relationship between Aminosugars in the Lipopolysaccharide, Serotype, and Aeruginocin Sensitivity in Strains of *Pseudomonas aeruginosa*

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INTRODUCTION

In a survey of the strains of *Pseudomonas aeruginosa* used in the Habs (1957) serotyping system we correlated aminocompound content of the lipopolysaccharides (LPS) with serotype (Chester, Meadow & Pitt, 1973). All the LPS contained alanine, glucosamine and galactosamine, but many could be distinguished by the presence of additional aminocompounds. These were detected by automatic aminocompound analysis, designated U1 and U8, but not further identified. We suggested that U3 might be quinovosamine and U4 fucosamine, but without markers were unable to confirm this. We have now re-examined the Habs type strains and a few others to identify the major unknown compounds. We have also tested their aeruginocin (pyocin) sensitivity since Suzuki (1974) reported that sensitivity of *P. aeruginosa* to the aeruginocin from strain P16 is correlated with the presence of quinovosamine in the LPS.

METHODS

Pseudomonas aeruginosa strains. The Habs serotyping strains 1 to 13 and strain PAC1 were described previously (Chester *et al.* 1973). Strains NCTC7244, NCTC8505, NCTC8203, NCTC8060, NCTC10332 and NCIB8626 were those used by Wilkinson & Galbraith (1975). Strains P14 and P16 were those described by Suzuki (1974). Strain PAC605 was a spontaneous mutant resistant to P16 aeruginocin. The aeruginocin preparations induced the lytic cycle of the lysogenic bacteriophage PSI carried by PAC1. Strain PAC605 was therefore derived from a spontaneous mutant of PAC1 resistant to PSI. This mutant, PAC1R, was otherwise indistinguishable from PAC1 in all properties tested including serotype, bacteriophage and aeruginocin sensitivity, and LPS structure (Chester & Meadow, 1975).

Growth of bacteria and analysis of lipopolysaccharides. Bacteria were grown, LPS isolated and aminocompounds detected as described by Chester *et al.* (1973). Fucosamine and quinovosamine were isolated from P16 and P14 LPS respectively by ion exchange chromatography on Dowex 50 (Wheat, 1966). Their identification was confirmed by paper chromatography in solvents A and B (Chester *et al.* 1973) and automatic aminocompound analysis.

Aeruginocin preparation and sensitivity. Crude aeruginocin preparations were made from P16 by mitomycin induction and titrated by doubling dilutions as described by Govan (1974), except that the growth medium was nutrient broth (Oxoid No. 2). Titres of 1:64 were obtained. Sensitivity was determined on nutrient agar plates. A loopful of the aeruginocin was streaked on the plate and allowed to dry. Cultures to be tested were grown for 4 h at 37 °C in nutrient broth and a loopful of each cross-streaked against the aeruginocin. Plates were incubated at 37 °C and inspected after 6 and 18 h.

Table 1. *The aminosugar composition of the lipopolysaccharide and the sensitivity to P16 aeruginocin of strains of P. aeruginosa of different serotypes*

The lipopolysaccharides were isolated, analysed and aeruginocin sensitivity determined as described in Methods. U1, 5, 6, 7, 8, X and Y are unidentified aminocompounds.

Strain	Habs serotype	Aminocompounds in LPS				Sensitivity to P16 aeruginocin
		2-Amino-2-deoxy-galacturonic acid	Quino-vosamine	Fucosamine	Others	
I	I	o	+	+	U5, 6, 7	S
P14	I	o	++	++	U5, 6, 7	S
NCTC7244	I	o*	+	+	.	S
NCIB8626	I	o*	+	+	.	R
2A	2A	o	o	tr	Y	R
2B	2B	o	o	tr	Y	R
3	3	+	o	tr	Y	S
PACI	3	+	o	tr	Y	S
NCTC8505	3	+	o*	o*	.	S
4	4	o	+	+	.	S
5C	5C	o	o	+	U8	R
NCTC8060	5C	o*	o*	+	.	R
5D	5D	+	o	+	.	R
6	6	+	tr	tr	U1, X, U5, 6, 7	S
NCTC10332	6	+	tr*	tr*	.	S
7	7	o	o	+	.	R
8	8	o	o	++	.	R
9	9	o	++	++	.	S
10	10	+	+	tr	.	S
11	11	o	o	++	.	R
P16	11	o	o	++	.	R
NCTC8203	11	o*	o*	+	.	R
12	12	o	+	tr	.	R
13	13	tr	+	tr	U5, 6, 7	R
PAC605	NT	tr	o	o	.	R

o, Not detected; tr, less than 1.0% (w/w); +, 1 to 5% (w/w); ++, 5% (w/w); NT, not typable; s, sensitive; R, resistant.

* Data taken from Wilkinson & Galbraith (1975).

RESULTS AND DISCUSSION

Aminosugar composition of the lipopolysaccharides

The most common of the eight unidentified aminocompounds previously described (Chester *et al.* 1973) was U4, which we suggested might be fucosamine. When the hydrolysed lipopolysaccharides were analysed with added fucosamine, the latter co-chromatographed with U4 in all strains except type 6. This LPS contained small amounts of fucosamine but the major peak in this region of the automatic analysis (3.5%, w/w, LPS) was eluted after fucosamine and clearly separated from it. It was not detected in any other LPS and has been designated X in Table 1. The fucosamine from PACI was isolated by ion exchange chromatography and shown to co-chromatograph with fucosamine in solvents A and B. Wilkinson and his colleagues detected fucosamine in LPS isolated from ten of the 12 strains they analysed (Wilkinson & Galbraith, 1975; Drewry *et al.* 1975), and Suzuki (1974) reported fucosamine in 17 of the 22 strains of *P. aeruginosa* examined; small amounts of fucosamine may have been present in the remaining five strains since she analysed whole bacteria not isolated

LPS. It seems likely therefore that fucosamine occurs in the LPS of most if not all strains of *P. aeruginosa*. However, the amount varies between strains and, as suggested by degradation studies, its position in the LPS may also vary (Chester *et al.* 1973; Chester & Meadow, 1975; Wilkinson & Galbraith, 1975; Drewry *et al.* 1975).

In hydrolysates of most of the LPS previously reported to contain U₃, this co-chromatographed with added quinovosamine (Table 1). However, automatic aminocompound analysis of the LPS from strains 2A, 2B, 3 and PAC1 (serotype 3) showed small amounts of an aminocompound eluting between quinovosamine and fucosamine but no quinovosamine. The amounts present were too small to be detected after ion exchange chromatography of hydrolysed LPS (50 mg). The compound has not been identified and is designated Y in Table 1. The absence of quinovosamine in the above strains may be related to their serotype since there was no quinovosamine in the LPS from any of the five strains of serotype 2B or of the strain of serotype 3 studied by Wilkinson & Galbraith (1975). Quinovosamine appears to be part of the high molecular weight fractions released by acetic acid hydrolysis of LPS. These fractions react specifically with homologous antisera and may correspond to side chains (Chester *et al.* 1973; Wilkinson & Galbraith, 1975).

Another aminocompound detected in LPS from several strains was U₂. In the automatic aminocompound analysis it runs close to diaminopimelic acid, which is sometimes a minor contaminant of LPS. However, U₂ could be separated from diaminopimelic acid by paper chromatography and had a much lower absorbance at 440 nm. A compound behaving like U₂ has been isolated from *P. aeruginosa* NCTC8505 LPS and identified as 2-amino-2-deoxygalacturonic acid (Wilkinson & Welbourn, 1975). The U₂ from PAC1 LPS was indistinguishable from this compound by paper chromatography, electrophoresis and automatic aminocompound analysis (S. G. Wilkinson, personal communication). We found 2-amino-2-deoxygalacturonic acid in LPS of all strains of serotypes 3, 5D, 6, 10 and 13 so far analysed (Table 1). We had previously reported that strain 1 also contained U₂, but we have not found any in later batches of LPS from the same strain and must conclude that the first analysis was incorrect. It is not clear what role, if any, 2-amino-2-deoxygalacturonic acid plays in the Habs serotyping system. Like quinovosamine it is concentrated in the high molecular weight fractions of the LPS. However, mutant PAC605, which no longer reacted with specific antiserum, retained about 10% of the aminogalacturonic acid of its parent strain PAC1.

Aeruginocin sensitivity

In a survey of 22 strains of *P. aeruginosa*, Suzuki (1974) found that those containing quinovosamine were sensitive to aeruginocins from P1 and P16. Of the strains we studied most of those sensitive to P16 aeruginocin contained quinovosamine in the LPS, while most of the resistant strains lacked this aminosugar (Table 1). However, despite their sensitivity to the aeruginocin, none of the three serotype 3 strains contained quinovosamine whereas three quinovosamine-containing strains (NCIB8626, 12 and 13) were aeruginocin resistant. The resistance of some quinovosamine-containing strains to P16 aeruginocin does not necessarily refute Suzuki's hypothesis that the aeruginocin receptor contains quinovosamine, since in tolerant strains absorption of the aeruginocin is not followed by lysis (Holloway *et al.* 1973). The test for sensitivity we used does not distinguish between tolerance and resistance.

The sensitivity of the serotype 3 strains to the aeruginocin was unexpected, but none of the strains examined by Suzuki were of this serotype (N. Suzuki, personal communication). It was not a characteristic of all serotype 3 strains since, out of six such isolates obtained from different clinical sources in the Cross Infection Reference Laboratory, Colindale, London, only two were sensitive to the aeruginocin. The type-3 LPS is one of the most com-

plex (Chester *et al.* 1973; Chester & Meadow, 1975; Wilkinson & Galbraith, 1975) and this may make these strains more aeruginocin sensitive. We have so far been unable to correlate the sensitivity of PAC1 to the P16 aeruginocin with any single component of the LPS. However, LPS is probably involved in sensitivity since the spontaneous mutant resistant to P16 aeruginocin, PAC605, has a polysaccharide-defective LPS (Koval & Meadow, 1975). Although this mutant retained 10% of the 2-amino-2-deoxygalacturonic acid of the parent strain, it lacked compound X, fucosamine and the basic 440 nm-absorbing compound (Chester & Meadow, 1975) found in small amounts in the parental LPS. Therefore in PAC1, resistance to P16 aeruginocin appears to be related to changes in LPS but not to quinovosamine. It thus differs from strain P14 in which a mutant resistant to P15 aeruginocin and also to the P16 aeruginocin had lost the quinovosamine in the LPS. Until we know more of the variation in structure between LPS from different strains of *P. aeruginosa* it is difficult to explain these differences.

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